

IN THE SPECIFICATION:

On page 1, please add the following paragraph underneath of the title:

CROSS-REFERENCE TO RELATED APPLICATION

This application is a divisional of application Serial No. 08/945,722, filed December 18, 1997, which is a 371 of PCT/GB96/01075 filed May 3, 1996.

Please delete the paragraph beginning on page 4, line 9 and replace it with the following paragraph:

Preferably the nucleotide sequence encodes a polypeptide comprising an effective portion of the amino acid sequence shown in Figure 5 (excluding the sequence MNKRIDL, **(SEQ ID No: 43)** which does not represent part of the SBE molecule), or a functional equivalent thereof (which term is discussed below). The amino acid sequence shown in Figure 5 (Seq ID No. 15) includes a leader sequence which directs the polypeptide, when synthesized in potato cells, to the amyloplast. Those skilled in the art will recognise that the leader sequence is removed to produce a mature enzyme and that the leader sequence is therefore not essential for enzyme activity. Accordingly, an "effective portion" of the polypeptide is one which possesses sufficient SBE activity to complement the branching enzyme mutation in *E. coli* KV 832 cells (described below) and which is active when expressed in *E. coli* in the phosphorylation stimulation assay. An example of an incomplete polypeptide which nevertheless constitutes an "effective portion" is the mature enzyme lacking the leader sequence. By analogy with the pea class A SBE sequence, the potato class A sequence shown in Figure 5 probably possesses a leader sequence of about 48 amino acid residues, such that the N terminal amino acid sequence is thought to commence around the glutamic acid residue (E) at position 49 **56** (EKSSYN...etc. **residues 56-61 of SEQ ID No: 15)**. Those skilled in the art will appreciate that an effective portion of the enzyme may well omit other parts of the sequence shown in the figure without substantial detrimental effect. For example, the C-terminal glutamic acid-rich region could be reduced in length, or possibly deleted entirely, without abolishing class A SBE activity. A comparison with

other known SBE sequences, especially other class A SBE sequences (see for example, Burton *et al*, 1995 cited above), should indicate those portions which are highly conserved (and thus likely to be essential for activity) and those portions which are less well conserved (and thus are more likely to tolerate sequence changes without substantial loss of enzyme activity).

Please delete the paragraph beginning on page 5, line 25, and replace it with the following paragraph:

The invention further provides a class A SBE polypeptide, obtainable from potato plants. In particular the invention provides the polypeptide in substantially pure form, especially in a form free from other plant-derived (especially potato plant-derived) components, which can be readily accomplished by expression of the relevant nucleotide sequence in a suitable non-plant host (such as any one of the yeast strains routinely used from expression purposes, e.g. *Pichia spp.* or *Saccharomyces spp.*). Typically the enzyme will substantially comprise the sequence of amino acid residues 49 to 882 (**i.e., amino acid residues 56 to 889 of SEQ ID No: 15**) shown in Figure 5 (disregarding the sequence MNKRIDL, **SEQ ID No: 43**, which is not part of the enzyme), or a functional equivalent thereof. The polypeptide of the invention may be used in a method of modifying starch *in vitro*, comprising treating starch under suitable conditions (e.g. appropriate temperature, pH, etc) with an effective amount of the polypeptide according to the invention.

Please delete the paragraph beginning on page 11, line 23, and replace it with the following paragraph:

Figure 4a shows the amino acid alignment of the C-terminal portion of starch branching enzyme isoforms from various sources (**SEQ ID Nos 21-26, respectively in order of appearance**): amino acid residues matching the consensus sequence are shaded;

Please delete the paragraph beginning on page 11, line 15, and replace it with the following paragraph:

Figure 4b shows the alignment of DNA sequences of various starch branching enzyme isoforms which encode a conserved amino acid sequence **(SEQ ID Nos 27-32, respectively in order of appearance)**;

Please delete the paragraph beginning on page 12, line 1, and replace it with the following paragraph:

Figure 6 shows a comparison of the most highly conserved part of the amino acid sequences of potato class A (uppermost sequence, **SEQ ID No: 33**) and class B (lowermost sequence, **SEQ ID No: 34**) SBE molecules;

Please delete the paragraph beginning on page 12, line 3, and replace it with the following paragraph:

Figure 7 shows a comparison of the amino acid sequence of the full length potato class A (uppermost sequence, **SEQ ID No: 35**) and pea (lowermost sequence, **SEQ ID No: 36**) class A SBE molecules;

Please delete the paragraph beginning on page 12, line 5, and replace it with the following paragraph:

Figure 8 shows a DNA alignment of various full length potato class A SBE clones obtained by the inventors **(SEQ ID No: 13, residues 1-3012 of SEQ ID Nos: 14, 12 and 18, respectively in order of appearance)**;

Please delete the paragraph beginning on page 12, line 6, and replace it with the following paragraph:

Figure 9 shows the DNA sequence of a potato class A SBE clone determined by direct sequencing of PCR products (SEQ ID No: 37), together with the predicted amino acid sequence (SEQ ID No: 38);

Please delete the paragraph beginning on page 12, line 8, and replace it with the following paragraph:

Figure 10 is a multiple DNA alignment of various full length potato SBE A clones obtained by the inventors (SEQ ID Nos: 39-41 and 16-17, respectively in order of appearance);

Please delete the paragraph beginning on page 12, line 10, and replace it with the following paragraph:

Figure 12 shows the DNA sequence (SEQ ID No: 19) and predicted amino acid sequence (SEQ ID No: 42) of the full length potato class A SBE clone as present in the plasmid pSJ90; and

Please delete the paragraph beginning on page 12, line 17, and replace it with the following paragraph:

The strategy for cloning the second form of starch branching enzyme from potato is shown in Figure 3. The small arrowheads represent primers used by the inventors in PCR and RACE protocols. The approximate size of the fragments isolated is indicated by the numerals on the right of the Figure. By way of explanation, a comparison of the amino acid sequences of several cloned plant starch branching enzymes (SBE) from maize (class A), pea (class A), maize (class B), rice (class B) and potato (class B), as well as human glycogen branching enzyme, allowed the inventors to identify a region in the carboxy-terminal one third of the protein which is almost completely conserved (GYLNFMGNEFGHPEWIDFPR, (residues 27-46 of SEQ ID No: 21 in

Figure 4a). A multiple alignment of the DNA sequences (human, pea class A, potato class B, maize class B, maize class A and rice class B, respectively) corresponding to this region is shown in Figure 4b and was used to design an oligo which would potentially hybridize to all known plant starch branching enzymes: AATTT(C/T)ATGGGIAA(C/T)GA(A/G)TT(C/T)GG (Seq ID No. 20).

Please delete the paragraph beginning on page 15, line 33, and replace it with the following paragraph:

Sequence analysis of the other two full length class A SBE genes showed that they contain frameshift mutations and are therefore unable to encode full length proteins and indeed they were unable to complement the branching enzyme deficiency in the KV832 mutant (described below). An alignment of the full length DNA sequences is shown in Figure 8: "10con.seq" (Seq ID No. 12), "19con.seq" (~~Seq ID No. 14~~residues 1-3012 of SEQ ID No: 14) and "11con.seq" (Seq ID No. 13) represent the sequence of full length clones 10, 19 and 11 obtained by PCR using the PBER1 and PBERT primers (see below), whilst "psbe2con.seq" (Seq ID No. 18) represents the consensus sequence of the RACE clones and cDNA clone 3.2.1. Those nucleotides which differ from the overall consensus sequence (not shown) are shaded. Dashes indicate gaps introduced to optimise the alignment. Apart from the frameshift mutations these clones are highly homologous. It should be noted that the 5' sequence of psbe2con is longer because this is the longest RACE product and it also contains several changes compared to the other clones. The upstream methionine codon is still present in this clone but the upstream ORF is shortened to just 3 amino acids and in addition there is a 10 base deletion in the 5' untranslated leader.

Please replace page 21 of the specification with the following pages, numbered 21a through 21e:

Please delete the sequence listing on pages 26 to 49 and renumber pages 50 to 56 accordingly as pages 26 to 32.